PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	David Nathan Abraham Fox, et al.	**Examiner:**	Unassigned
Serial No:	Unassigned	**Art Unit:**	Unassigned
Filed:	Herewith	**Docket:**	16789 (PC25204)
For:	NOVEL COMBINATION	**Dated:**	June 25, 2003

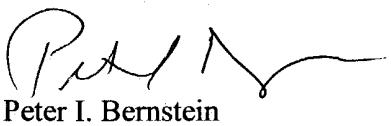
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CLAIM OF PRIORITY

Sir:

Applicants in the above-identified application hereby claim the right of priority in connection with Title 35 U.S.C. § 119 and in support thereof, herewith submit a certified copy of United Kingdom Patent Application No. 0214784.1, filed on June 26, 2002.

Respectfully submitted,



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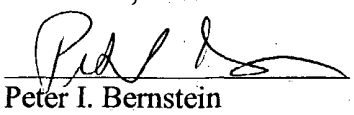

Peter I. Bernstein

The Patent Office
Concept House
Cardiff Road
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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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Signed Andrew Gersey

Dated 1 April 2003

Patents Form 1/77

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

PCS25204DAK-PROV

2. Patent application number
(The Patent Office will fill in this part)

0214784.1

26 JUN 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

PFIZER LIMITED
Ramsgate Road,
Sandwich,
Kent, CT13 9NJ

Patents ADP number *(if you know it)*

United Kingdom

If the applicant is a corporate body, give the country/state of its incorporation

5060102000 1

4. Title of the invention

NOVEL COMBINATION

5. Name of your agent *(if you have one)*

Dr. David A. Kendrick

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

UK Patent Department
Ramsgate Road,
Sandwich, Kent,
CT13 9NJ
United Kingdom

8412160001

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	18
Claim *(s)*	5 DML
Abstract	1
Drawing *(s)*	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Dr. David A. Kendrick Date 26 June 2002

12. Name and daytime telephone number of person to contact in the United Kingdom 44 1304 646010

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

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Nov l Combinati n

The invention relates to a combination of a) an inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) inhibitor and b) an angiotensin II receptor antagonist and particularly the use of such a combination for treating hypertension.

Blood pressure (BP) is defined by a number of haemodynamic parameters taken either in isolation or in combination. Systolic blood pressure (SBP) is the peak arterial pressure attained as the heart contracts. Diastolic blood pressure is the minimum arterial pressure attained as the heart relaxes. The difference between the SBP and the DBP is defined as the pulse pressure (PP).

Hypertension, or elevated BP, has been defined as a SBP of at least 140mmHg and/or a DBP of at least 90mmHg. By this definition, the prevalence of hypertension in developed countries is about 20% of the adult population, rising to about 60-70% of those aged 60 or more, although a significant fraction of these hypertensive subjects have normal BP when this is measured in a non-clinical setting. Some 60% of this older hypertensive population have isolated systolic hypertension (ISH), i.e. they have an elevated SBP and a normal DBP. Hypertension is associated with an increased risk of stroke, myocardial infarction, atrial fibrillation, heart failure, peripheral vascular disease and renal impairment (Fagard, RH; Am. J. Geriatric Cardiology 11(1), 23-28, 2002; Brown, MJ and Haycock, S; Drugs 59(Suppl 2), 1-12, 2000).

The pathophysiology of hypertension is the subject of continuing debate. While it is generally agreed that hypertension is the result of an imbalance between cardiac output and peripheral vascular resistance, and that most hypertensive subjects have normal cardiac output and increased peripheral resistance there is uncertainty which parameter changes first (Beevers, G *et al.*; BMJ 322, 912-916, 2001).

Despite the large number of drugs available in various pharmacological categories, including diuretics, alpha-adrenergic antagonists, beta-adrenergic antagonists, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor antagonists, the need for an effective treatment of hypertension is still not satisfied.

Angiotensin II receptor antagonists (angiotensin receptor blockers, ARBs), which block the vasoconstrictive action of the renin-angiotensin-aldosterone system, are generally considered to be more selective than angiotensin converting enzyme inhibitors, which work on the same physiological pathway, and to produce fewer side effects.

Phosphodiesterase type 5 (PDE5) is a cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase. Inhibitors of PDE5 decrease the rate of hydrolysis of cGMP and so potentiate the actions of nitric oxide. They have been suggested as antihypertensive agents but have not yet been adopted as therapeutic agents in this field. They are, however, useful in the treatment of male erectile dysfunction.

According to a first aspect, the present invention provides the use of a combination comprising a) a PDE5 inhibitor and b) an angiotensin II receptor antagonist in the manufacture of a medicament for treating hypertension. The term "hypertension" includes all diseases characterised by supranormal blood pressure, such as essential hypertension, pulmonary hypertension, secondary hypertension, isolated systolic hypertension, hypertension associated with diabetes, hypertension associated with atherosclerosis, and renovascular hypertension. The term "treating hypertension" includes the palliative, curative and prophylactic treatment of hypertension, complications arising from hypertension, and other associated co-morbidities, including congestive heart failure, angina, stroke and the like.

Hereinafter combinations of a PDE5 inhibitor and an angiotensin II receptor antagonist, including combinations of specific PDE5 inhibitors and specific

angiotensin II receptor antagonists, will be referred to as combinations of the invention.

The combinations of the invention have the advantage that they are more potent, less toxic or have other more desirable properties than PDE5 inhibitors or angiotensin II receptor antagonists when used alone for treating hypertension.

Hereinafter the term "the PDE5 inhibitor" means a PDE5 inhibitor for use in the invention, including all pharmaceutically acceptable salts, solvates and polymorphs of that PDE5 inhibitor. Similarly, the term the term "the angiotensin II receptor antagonist" means an angiotensin II receptor antagonist for use in the invention, including all pharmaceutically acceptable salts, solvates and polymorphs of that angiotensin II receptor antagonist.

The suitability of the PDE5 inhibitor and the angiotensin II receptor antagonist can be readily determined by evaluation of their potency and selectivity using literature methods followed by evaluation of their toxicity, pharmacokinetics (absorption, metabolism, distribution and elimination), etc in accordance with standard pharmaceutical practice. Suitable compounds are those that are potent and selective, have no significant toxic effect at the therapeutic dose, and preferably are bioavailable following oral administration.

Potency can be defined as an IC_{50} value, being the concentration of compound necessary to inhibit the enzyme activity by 50%. IC_{50} values for the PDE5 inhibitors may be determined using the PDE5 assay in the Test Methods Section hereinafter. Preferably, the PDE5 inhibitors have an IC_{50} against the PDE5 enzyme of less than 100nM, more preferably less than 50nM.

Selectivity ratios may readily be determined by the skilled person, by ratio of corresponding IC_{50} values for the particular enzymes concerned. IC_{50} values for the

PDE3 and PDE4 enzyme may be determined using established literature methodology, see Ballard SA *et al.*; Journal of Urology 159, 2164-2171, 1998.

Preferably the PDE5 inhibitors are selective for the PDE5 enzyme. Preferably they have a selectivity for PDE5 over PDE3 of greater than 100, more preferably greater than 300. More preferably the PDE5 has a selectivity over both PDE3 and PDE4 of greater than 100, more preferably greater than 300.

Preferably the PDE5 inhibitors have an IC_{50} against PDE5 of less than 100nM and a selectivity over PDE3 of greater than 100 fold.

Oral bioavailablity refers to the proportion of an orally administered drug that reaches the systemic circulation. The factors that determine oral bioavailability of a drug are dissolution, membrane permeability and hepatic clearance. Typically, a screening cascade of firstly *in vitro* and then *in vivo* techniques is used to determine oral bioavailablity.

Dissolution, the solubilisation of the drug by the aqueous contents of the gastro-intestinal tract (GIT), can be predicted from in vitro solubility experiments conducted at appropriate pH to mimic the GIT. Preferably the PDE5 inhibitors have a minimum solubility of 50μg/ml. Solubility can be determined by standard procedures known in the art such as described in Lipinski CA et al.; Adv. Drug Deliv. Rev. 23(1-3), 3-25, 1997.

Membrane permeability refers to the passage of a compound through the cells of the GIT. Lipophilicity is a key property in predicting this and is determined by *in vitro* Log $D_{7.4}$ measurements using organic solvents and buffer. Preferably the PDE5 inhibitors have a Log $D_{7.4}$ of -2 to +4, more preferably -1 to +3. The Log D can be determined by standard procedures known in the art such as described in Stopher, D and McClean, S; J. Pharm. Pharmacol. 42(2), 144, 1990.

Cell monolayer assays such as Caco2 add substantially to prediction of favourable membrane permeability in the presence of efflux transporters such as P-glycoprotein, so-called Caco2 flux. Preferably, the PDE5 inhibitors have a Caco2 flux of greater than $2x10^{-6}cms^{-1}$, more preferably greater than $5x10^{-6}cms^{-1}$. The Caco2 flux value can be determined by standard procedures known in the art such as described in Artursson, P and Magnusson, C; J. Pharm. Sci, 79(7), 595-600, 1990.

Metabolic stability addresses the ability of the GIT to metabolise compounds during the absorption process or the liver to do so immediately post-absorption: the first pass effect. Assay systems such as microsomes, hepatocytes etc are predictive of metabolic lability. Preferably the PDE5 inhibitors show metabolic stability in the assay system that is commensurate with an hepatic extraction of less then 0.5. Examples of assay systems and data manipulation are described in Obach, RS; Curr. Opin. Drug Disc. Devel. 4(1), 36-44, 2001 and Shibata, Y *et al.*; Drug Met. Disp. 28(12), 1518-1523, 2000.

Because of the interplay of the above processes, further support that a drug will be orally bioavailable in humans can be gained by *in vivo* experiments in animals. Absolute bioavailability is determined in these studies by administering the compound separately or in mixtures by the oral route. For absolute determinations (% orally bioavailable) the intravenous route is also employed. Examples of the assessment of oral bioavailability in animals can be found in Ward, KW *et al.*; Drug Met. Disp. 29(1), 82-87, 2001; Berman, J *et al.*; J. Med. Chem. 40(6), 827-829, 1997 and Han KS and Lee, MG; Drug Met. Disp. 27(2), 221-226, 1999.

Examples of PDE5 inhibitors for use with the invention are:

The pyrazolo[4,3-*d*]pyrimidin-7-ones disclosed in EP-A-0463756, EP-A-0526004 and published international patent applications WO 93/06104, WO 98/49166, WO 99/54333, WO 00/24745, WO 01/27112 and WO 01/27113; the pyrazolo[3,4-

d]pyrimidin-4-ones disclosed in EP-A-0995750, EP-A-0995751 and published international patent application WO 93/07149; the quinazolin-4-ones disclosed in published international patent application WO 93/12095; the pyrido[3,2-*d*]pyrimidin-4-ones disclosed in published international patent application WO 94/05661; the purin-6-ones disclosed in EP-A-1092718 and in published international patent application WO 94/00453; the hexahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indole-1,4-diones disclosed in published international application WO 95/19978; the imidazo[5,1-*f*][1,2,4]triazin-ones disclosed in EP-A-1092719 and in published international application WO 99/24433; the bicyclic compounds disclosed in published international application WO 93/07124 and the imidazoquinazolinones disclosed in Rotella DP *et al*; J. Med. Chem. 43(7), 1257-1263, 2000.

The contents of the published patent applications and journal articles and in particular the general formulae of the therapeutically active compounds of the claims and exemplified compounds therein are incorporated herein in their entirety by reference thereto.

Still further examples of PDE5 inhibitors for use with the invention include: 4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2*H*)pyridazinone; 1-[4-[(1,3-benzodioxol-5- ylmethyl)amiono]-6-chloro-2-quinozolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-*cis*-5,6a,7,9,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-4,5]imidazo[2,1-b]purin-4(3*H*)one; furazlocillin; *cis*-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a- octahydrocyclopent[4,5]-imidazo[2,1-*b*]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl) propoxy)-3-(2*H*)pyridazinone; l-methyl-5(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro-7*H*-pyrazolo(4,3-*d*)pyrimidin-7-one; 1-[4-[(1,3-benzodioxol-5-ylmethyl)arnino]-6-chloro-2- quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt; Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; see WO 96/26940);

Pharmaprojects No. 5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 and E-4010 (Eisai); Bay-38-3045 & 38-9456 (Bayer) and Sch-51866.

Preferred PDE5 inhibitors for use with the invention include:

5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil) also known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine (see EP-A-0463756);

5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see EP-A-0526004);

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO98/49166);

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO99/54333);

(+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(*R*)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-*d*]pyrimidin-7-one, also known as 3-ethyl-5-{5-[4-ethylpiperazin-1-ylsulphonyl]-2-([(1*R*)-2-methoxy-1-methylethyl]oxy)pyridin-3-yl}-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO99/54333);

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one, also known as 1-{6-ethoxy-5-[3-ethyl-6,7-dihydro-2-(2-methoxyethyl)-7-oxo-2*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl]-3-pyridylsulphonyl}-4-ethylpiperazine (see WO 01/27113, Example 8);

5-[2-*iso*-butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO 01/27113, Example 15);

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO 01/27113, Example 66);

5-(5-acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO 01/27112, Example 124);

5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see WO 01/27112, Example 132);

(6*R*,12a*R*)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-*b*]indole-1,4-dione (tadalafil, IC-351), i.e. the compound of examples 78 and 95 of published international application WO95/19978, as well as the compound of examples 1, 3, 7 and 8;

2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-*f*][1,2,4]triazin-4-one (vardenafil) also known as 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-*f*]-as-triazin-2-yl)-4-ethoxyphenyl]sulphonyl]-4-ethylpiperazine, i.e. the compound of examples 20, 19, 337 and 336 of published international application WO99/24433;

4-(4-chlorobenzyl)amino-6,7,8-trimethoxyquinazoline (example 11 of published international application WO93/07124 (EISAI)); and

7,8-dihydro-8-oxo-6-[2-propoxyphenyl]-1*H*-imidazo[4,5-*g*]quinazoline and 1-[3-[1-[(4-fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]carboxamide (compounds 3 and 14 from Rotella DP *et al.*; J. Med. Chem. 43(7), 1257-1263, 2000).

More preferred PDE5 inhibitors for use with the invention are selected from the group and pharmaceutically acceptable salts thereof:
5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil);
(6*R*,12a*R*)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-*b*]indole-1,4-dione (tadalafil, IC-351);
2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-one (vardenafil);
5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one; and
5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one.

A particularly preferred PDE5 inhibitor is 5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil) (also known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine) and pharmaceutically acceptable salts thereof. Sildenafil citrate is a preferred salt.

Examples of angiotensin II receptor antagonists for use with the invention include candesartan, eprosartan, irbesartan, losartan, olmesartan, olmesartan medoxomil, saralasin, telmisartan and valsartan.

Preferred combinations of PDE5 inhibitors and angiotensin II receptor antagonists for treating hypertension are:

sildenafil and candesartan;
sidenafil and eprosartan;
sildenafil and irbesartan;

sildenafil and losartan;
sildenafil and olmesartan;
sildenafil and olmesartan medoxomil;
sildenafil and telmisartan;
sildenafil and valsartan;
tadalafil and candesartan;
tadalafil and eprosartan;
tadalafil and irbesartan;
tadalafil and losartan;
tadalafil and olmesartan;
tadalafil and olmesartan medoxomil;
tadalafil and telmisartan;
tadalafil and valsartan;
vardenafil and candesartan;
vardenafil and eprosartan;
vardenafil and irbesartan;
vardenafil and losartan;
vardenafil and olmesartan;
vardenafil and olmesartan medoxomil;
vardenafil and telmisartan; and
vardenafil and valsartan.

The combination of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the combinations of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, multi-particulates, gels, films, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release

applications. The combinations of the invention may also be administered as fast-dispersing or fast-dissolving dosage forms or in the form of a high energy dispersion or as coated particles. Suitable formulations may be in coated or uncoated form, as desired.

Such solid pharmaceutical compositions, for example, tablets, may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine and starch (preferably corn, potato or tapioca starch), disintegrants such as sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention. Active ingredient means a combination of the invention.

Formulation 1:

A tablet is prepared using the following ingredients :

Active ingredient (50mg) is blended with cellulose (microcrystalline), silicon dioxide, stearic acid (fumed) and the mixture is compressed to form tablets.

Formulation 2:

An intravenous formulation may be prepared by combining active ingredient (100mg) with isotonic saline (1000ml)

The tablets are manufactured by a standard process, for example, direct compression or a wet or dry granulation process. The tablet cores may be coated with appropriate overcoats.

Solid compositions of a similar type may also be employed as fillers in gelatin or HPMC capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the PDE5 inhibitor and angiotensin II receptor antagonist may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

Modified release and pulsatile release dosage forms may contain excipients such as those detailed for immediate release dosage forms together with additional excipients that act as release rate modifiers, these being coated on and/or included in the body of the device. Release rate modifiers include, but are not exclusively limited to, hydroxypropylmethyl cellulose, methyl cellulose, sodium carboxymethylcellulose, ethyl cellulose, cellulose acetate, polyethylene oxide, Xanthan gum, Carbomer, ammonio methacrylate copolymer, hydrogenated castor oil, carnauba wax, paraffin wax, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, methacrylic acid copolymer and mixtures thereof. Modified release and pulsatile release dosage forms may contain one or a combination of release rate modifying excipients. Release rate modifying excipients may be present both within the dosage form i.e. within the matrix, and/or on the dosage form, i.e. upon the surface or coating.

Fast dispersing or dissolving dosage formulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium, crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol, xylitol. The terms dispersing or dissolving as used herein to describe FDDFs are dependent upon the solubility of the drug substance used i.e. where the drug substance is insoluble a fast dispersing

dosage form can be prepared and where the drug substance is soluble a fast dissolving dosage form can be prepared.

The combinations of the invention can also be administered parenterally, for example, *intracavernouslly, intravenously, intra-arterially, intraperitoneally,* intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion or needleless injection techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

The following dosage levels and other dosage levels herein are for the average human subject having a weight range of about 65 to 70kg. The skilled person will readily be able to determine the dosage levels required for a subject whose weight falls outside this range, such as children and the elderly.

The dosage of the combination of the invention in such formulations will depend on its potency, *but can be expected to be in the range of from 1 to 500mg of PDE5* inhibitor and 1 to 300mg of angiotensin II receptor antagonist for administration up to three times a day. A preferred dose is in the range 10 to 100mg (e.g. 10, 25, 50 and 100mg) of PDE5 inhibitor and 20 to 150mg (e.g. 20, 50, 100 and 150mg) of angiotensin II receptor antagonist which can be administered once, twice or three times a day (preferably once). However the precise dose will be as determined by the prescribing physician and will depend on the age and weight of the subject and severity of the symptoms.

For oral and parenteral administration to human patients, the daily dosage level of a combination of the invention will usually be from to 5 to 500mg/kg (in single or divided doses).

Thus tablets or capsules may contain from 5mg to 250mg (for example 10 to 100mg) of the combination of the invention for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention. The skilled person will appreciate that the combinations of the invention may be taken as a single dose as needed or desired (i.e. prn). It is to be appreciated that all references herein to treatment include acute treatment (taken as required) and chronic treatment (longer term continuous treatment).

The combinations of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray, atomiser or nebuliser, with or without the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark]) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray, atomiser or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the combinations of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1μg to 50mg of a combination of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1μg to 50mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the combinations of the invention can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The combinations of the invention may also be dermally or transdermally administered, for example, by the use of a skin patch, depot or subcutaneous injection. They may also be administered by the pulmonary or rectal routes.

For application topically to the skin, the combinations of the invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The combinations of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or

solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in published international patent applications WO91/11172, WO94/02518 and WO98/55148.

Oral administration of the combinations of the invention is a preferred route, being the most convenient. In circumstances where the recipient suffers from a swallowing disorder or from impairment of drug absorption after oral administration, the drug may be administered parenterally, sublingually or buccally.

The combinations of the invention may be used as part of a triple therapy regimen, i.e. a treatment protocol in which the patient is treated with three pharmaceutical agents. The third agent in the triple therapy may be a second PDE5 inhibitor or angiotensin II receptor antagonist, or it may be chosen from a third pharmacological group. For example, it may be a neutral endopeptidase inhibitor, an angiotensin converting enzyme inhibitor, a calcium channel blocker such as amlodipine, a statin such as atorvastatin, a beta blocker (i.e. a beta-adrenergic receptor antagonist) or a diuretic.

It will be appreciated that the invention covers the following further aspects and that the embodiments specified hereinabove for the first aspect extend to these aspects:

i) a pharmaceutical combination of the invention (for simultaneous, separate or sequential administration) for treating hypertension;

ii) a kit for treating hypertension, the kit comprising: a) a first pharmaceutical composition comprising a PDE5 inhibitor; b) a second pharmaceutical composition comprising an angiotensin II receptor antagonist; and c) a container for the compositions;

iii) a method of treating hypertension in a subject comprising treating said patient with an effective amount of a combination of the invention.

The pharmaceutical combination of the invention may also be useful in the treatment of other diseases, such as diabetes, glaucoma and thrombosis, and in the management of patients following percutaneous translumenal coronary angioplasty ("post-PTCA patients").

Assay

Preferred compounds suitable for use in accordance with the present invention are potent and selective PDE5 inhibitors. *In vitro* PDE inhibitory activities against cyclic guanosine 3',5'-monophosphate (cGMP) and cyclic adenosine 3',5'-monophosphate (cAMP) phosphodiesterases can be determined by measurement of their IC_{50} values (the concentration of compound required for 50% inhibition of enzyme activity).

The required PDE enzymes can be isolated from a variety of sources, including human corpus cavernosum, human and rabbit platelets, human cardiac ventricle, human skeletal muscle and bovine retina, essentially by the method of Thompson WJ and Appleman MM; Biochemistry 10(2),311-316, 1971. In particular, the cGMP-specific PDE (PDE5) and the cGMP-inhibited cAMP PDE (PDE3) can be obtained from human corpus cavernosum tissue, human platelets or rabbit platelets; the cGMP-stimulated PDE (PDE2) was obtained from human corpus cavernosum; the calcium/calmodulin (Ca/CAM)-dependent PDE (PDE1) from human cardiac ventricle; the cAMP-specific PDE (PDE4) from human skeletal muscle; and the photoreceptor PDE (PDE6) from bovine retina. Phosphodiesterases 7-11 can be generated from full length human recombinant clones transfected into SF9 cells.

Assays can be performed either using a modification of the "batch" method of Thompson, WJ *et al.*; Biochemistry 18(23), 5228-5237, 1979, or using a scintillation proximity assay for the direct detection of AMP/GMP using a modification of the protocol described by Amersham plc under product code TRKQ7090/7100. In summary, the effect of PDE inhibitors was investigated by assaying a fixed amount of enzyme in the presence of varying inhibitor concentrations and low substrate,

(cGMP or cAMP in a 3:1 ratio unlabelled to [^{3}H]-labeled at a concentration of ~1/3 K_m) such that $IC_{50} \cong K_i$. The final assay volume was made up to 100µl with assay buffer [20mM Tris-HCl pH 7.4, 5mM $MgCl_2$, 1mg/ml bovine serum albumin]. Reactions were initiated with enzyme, incubated for 30-60min at 30°C to give <30% substrate turnover and terminated with 50µl yttrium silicate SPA beads (containing 3mM of the respective unlabelled cyclic nucleotide for PDEs 9 and 11). Plates were re-sealed and shaken for 20min, after which the beads were allowed to settle for 30min in the dark and then counted on a TopCount plate reader (Packard, Meriden, CT) Radioactivity units were converted to % activity of an uninhibited control (100%), plotted against inhibitor concentration and inhibitor IC_{50} values obtained using the 'Fit Curve' Microsoft Excel extension.

Animal study

The efficacy of the combinations of the invention may be determined in the spontaneously hypertensive rat, which is a widely used model of human hypertension. Animals are instrumented with Doppler flow probes for the measurement of mesenteric, hindquarters and renal blood flow, aortic blood pressure and heart rate according to published methods (Gardiner, SM *et al.*; Br. J. Pharmacol. 132(8), 1625-1629, 2001). Baseline haemodynamic parameters are recorded. Animals (n=3/group) are then treated with an angiotensin II receptor antagonist alone, a PDE5 inhibitor alone, or with a combination of an angiotensin II receptor antagonist and a PDE5 inhibitor by continuous infusion over 80 hours. A control group of animals receives saline. Changes in haemodynamic parameters are monitored during the study period.

Claims

1 The use of a combination of an inhibitor of cyclic guanosine monophosphate specific phosphodiesterase type 5 (PDE5) and an angiotensin II receptor antagonist for the preparation of a medicament for the treatment of hypertension.

2 The use according to claim 1, wherein the inhibitor of PDE5 has an IC_{50} value of less than 100nM.

3 The use according to any preceding claim, wherein the inhibitor of PDE5 has an IC_{50} value of less than 50nM.

4 The use according to any preceding claim, wherein the inhibitor of PDE5 is selected from

5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil);

5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

(+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(*R*)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-[2-*iso*-butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-(5-acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

(6*R*,12a*R*)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-*b*]indole-1,4-dione (tadalafil);

2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-*f*][1,2,4]triazin-4-one (vardenafil);

4-(4-chlorobenzyl)amino-6,7,8-trimethoxyquinazoline;

7,8-dihydro-8-oxo-6-[2-propoxyphenyl]-1*H*-imidazo[4,5-*g*]quinazoline; and

1-[3-[1-[(4-fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]carboxamide

and pharmaceutically acceptable salts thereof.

The use according to any preceding claim, wherein the inhibitor of PDE5 is

selected from

5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil);

(6*R*,12a*R*)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-*b*]indole-1,4-dione (tadalafil);

2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-*f*][1,2,4]triazin-4-one (vardenafil);

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one; and

5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one

and pharmaceutically acceptable salts thereof.

6 The use according to any preceding claim, wherein the inhibitor of PDE5 is selected from 5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (sildenafil) and pharmaceutically acceptable salts thereof.

7 The use according to any preceding claim, wherein the inhibitor of PDE5 is sildenafil citrate.

8 The use according to any preceding claim, wherein the angiotensin II receptor antagonist is selected from candesartan, eprosartan, irbesartan, losartan, olmesartan, olmesartan medoxomil, saralasin, telmisartan and valsartan and pharmaceutically acceptable salts thereof.

9 The use according to any preceding claim, wherein the combination of the inhibitor of PDE5 and the angiotensin II receptor antagonist is selected from
sildenafil and candesartan;
sidenafil and eprosartan;
sildenafil and irbesartan;
sildenafil and losartan;
sildenafil and olmesartan;
sildenafil and olmesartan medoxomil;
sildenafil and telmisartan;
sildenafil and valsartan;
tadalafil and candesartan;
tadalafil and eprosartan;
tadalafil and irbesartan;
tadalafil and losartan;
tadalafil and olmesartan;
tadalafil and olmesartan medoxomil;
tadalafil and telmisartan;
tadalafil and valsartan;
vardenafil and candesartan;
vardenafil and eprosartan;
vardenafil and irbesartan;
vardenafil and losartan;
vardenafil and olmesartan;
vardenafil and olmesartan medoxomil;
vardenafil and telmisartan; and
vardenafil and valsartan.

10 The use according to any preceding claim, wherein the combination of the inhibitor of PDE5 and the angiotensin II receptor antagonist is selected from sildenafil citrate and candesartan;

sidenafil citrate and eprosartan;
sildenafil citrate and irbesartan;
sildenafil citrate and losartan;
sildenafil citrate and olmesartan;
sildenafil citrate and olmesartan medoxomil;
sildenafil citrate and telmisartan; and
sildenafil citrate and valsartan.

11 A pharmaceutical composition comprising an inhibitor of cyclic guanosine monophosphate specific phosphodiesterase type 5 (PDE5) and an angiotensin II receptor antagonist.

12 A pharmaceutical combination for simultaneous, separate or sequential administration for treating hypertension, comprising an inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) and an angiotensin II receptor antagonist.

13 A kit for treating hypertension, the kit comprising: a) a first pharmaceutical composition comprising a PDE5 inhibitor; b) a second pharmaceutical composition comprising an angiotensin II receptor antagonist; and c) a container for the compositions.

14 A method of treating hypertension in a subject comprising treating said patient simultaneously, separately or sequentially with an effective amount of an inhibitor of PDE5 and an angiotensin II receptor antagonist.

Novel Combination

ABSTRACT

Combinations comprising a) an inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) inhibitor and b) an angiotensin II receptor antagonist are useful for treating hypertension.